U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: OGATA4

U S APPLICATION NO (If known,see 37 CFR 1 5): 09/763370

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY CLAIMED
PCT/JP99/04480	20 August 1999	21 August 1998

TITLE OF INVENTION: METHOD FOR DIAGNOSING BONE METASTASIS OF MALIGNANT TUMOR

APPLICANT(S) FOR DO/EO/US: **E, OGATA et al.**

PATENT TRADEMARK OFFICE FEB 21 2001

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [xx] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [xx] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [xx] The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31).
5. [xx] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not transmitted by the International Bureau).
   b. [xx] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [xx] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [xx] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [xx] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [xx] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern *document(s)* or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [xx] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [xx] Other items or information:
    [xx] Courtesy copy of the first page of the International Publication (WO 00/11480).
    [xx] Courtesy copy of the International Preliminary Examination Report. There were no annexes.
    [xx] Formal drawings, 4 sheets, Figures 1-4.
    [xx] Courtesy Copy of the International Search Report.

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	International Application No	Attorney's Docket No
09/763370	PCT/JP99/04480	OGATA4

17. [xx] The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a)(1) –(5):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... ....**$1000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....**$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$710.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....**$690.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ]20 [ ]30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$
Claims as Originally Presented	Number Filed	Number Extra	Rate	
Total Claims	17 - 20		X $18.00	$ 0
Independent Claims	2 - 3		X $80.00	$ 0
Multiple Dependent Claims (if applicable)			+$270.00	$ 0
TOTAL OF ABOVE CALCULATIONS =				$ 860.00
Claims After Post Filing Prel. Amend	Number Filed	Number Extra	Rate	
Total Claims	- 20		X $18.00	$ 0
Independent Claims	- 3		X $78.00	$ 0
TOTAL OF ABOVE CALCULATIONS =				$ 860.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27.				$
SUBTOTAL =				$ 860.00
Processing fee of **$130.00** for furnishing the English translation later than [ ]20 [ ]30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 860.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$
TOTAL FEES ENCLOSED =				$ 860.00
			Amount to be: refunded	$
			charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [XX] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of $860.00, is attached.

c. [ ] Please charge my Deposit Account No. **02-4035** in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. [**XX**] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Date of this submission: **February 21, 2001**

SIGNATURE

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NAME

25,618
REGISTRATION NUMBER

09/763370
JC03 Rec'd PCT/PTO 21 FEB 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
E. OGATA et al.	)	
	)	
IA No.: PCT/JP99/04480	)	
	)	Washington, D.C.
IA Filed: August 20, 1999	)	
	)	
U.S. App. No.:	)	
(Not Yet Assigned)	)	
	)	February 21, 2001
National Filing Date:	)	
(Not Yet Received)	)	
	)	
For: METHOD FOR DIAGNOSING...	)	Docket No.: OGATA4

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

REFERENCE TO RELATED APPLICATIONS

The present application is the national stage under 35 U.S.C. §371 of international application PCT/JP99/04480, filed August 20, 1999, which designated the United States, and was not published in English. --

IN THE CLAIMS

Claim 3, line 1, delete "or 2".

Claim 4, line 1, change "any one of claims 1-3" to --claim 1--.

Claim 5, line 1, change "any one of claims 1-4" to --claim 1--.

Claim 6, line 1, change "any one of claims 1-5" to --claim 1--.

Claim 12, line 1, change "any one of claims 8-11" to --claim 8--.

Claim 13, line 1, change "any one of claims 8 or 12" to --claim 8--.

Claim 14, line 1, change "any one of claims 8-13" to --claim 8--.

Claim 15, line 1, change "any one of claims 8-14" to --claim 8--.

Claim 16, line 1, change "any one of claims 8-15" to --claim 8--.

REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage. The above amendments to the claims are being made in order to eliminate any properly multiply dependent claims, for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
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4 PAGES

09/763370
JC03 Rec'd PCT/PTO 21 FEB 2001

METHOD OF DIAGNOSING BONE METASTASIS OF MALIGNANT TUMORS

TECHNICAL FIELD

This invention relates to a method of diagnosing bone metastasis accompanying malignant tumors such as breast cancer, prostatic cancer and lung cancer; the invention also relates to a method of evaluating the therapeutic effect of drugs used to treat these diseases.

BACKGROUND ART

Bone metastasis of cancer is conventionally diagnosed by examining clinical symptoms of the patient or images taken by simple radiography, bone scintigraphy, CT, MRI, etc. From a visual viewpoint, bone metastases are classified as a dissolution type, a hardening type or a mixed type depending on the balance between bone dissolution and formation at the site of bone metastasis. While image diagnoses are highly reliable and useful, they are generally too expensive to be used for screening and monitoring purposes.

With the recent advances in the study of bone metabolism, various markers have been developed as indices of bone metabolism. Markers of bone formation and resorption are separately listed in Table 1. Attempts are being made to diagnose bone metastases of cancers using those bone metabolic markers (Koizumi, M. et al., Bone Metabolic Markers in Bone Metastases, J. Cancer Res. and Clin. Oncol., 121:541-548, 1995).

Table 1

Markers of bone formation
(1) Type I procollagen peptides ..... proliferation
C-terminal propeptide (PICP)
N-terminal propeptide (PINP)
(2) Alkali phosphatases ..... matrix formation
total alkali phosphatase (ALP)
bone alkali phosphatase (BALP)
(3) Osteocalcin (OC) ..... mineralization
C-terminal fragments
intermediate portions
intact
Markers of bone resorption
(1) pyridinium cross-links
total urinary pyridinoline·deoxypyridinoline (HPLC method)
free urinary deoxypyridinoline (fDPD)
(2) pyridinium crosslinked collagen peptide fragments
serum C-terminal telopeptide (ICTP)
urinary C-terminal telopeptide (CTx)
urinary N-terminal telopeptide (NTx)
(3) Tartrate-resistant acid phosphatase (TRAP)
(4) Galactosyl hydroxylysine (GHYL)
(5) Hydroxyproline
(6) N-terminal osteocalcin

Most of the bone metabolic markers have as their rationale the measurement of metabolic products that are

released into blood and urine in the process of formation and absorption of type I collagen which accounts for 90% of the bone matrix. To be more specific, type I procollagen which is synthesized during bone formation releases C- and N-terminal propeptides when it is converted to type I collagen and these propeptides serve as markers of bone formation. In the process of bone resorption, the type I collagen in the bone matrix undergoes metabolism to be released into blood and urine; the measured blood and urine levels of the released type I collagen serve as markers of bone resorption.

Bone formation is known to consist of three major phases depending upon the stage of proliferation and differentiation of osteoblasts; the first phase is where oesteoblasts proliferate and the matrix forms, the second phase is for matrix maturation and the third phase is for calcification, and different markers are known to appear at different phases (Stein, G.S. et al.: Relationship of Cell Growth to the Regulation of Tissue-Specific Gene Expression during Osteoblast Differentiation, FASEB J., 4:3111-3123, 1990).

In the phase of osteoblast proliferation and matrix formation, type I collagen forms actively and C- and N-terminal propeptides appear in the blood. In the phase of matrix maturation, bone alkali phosphatase (BALP) is generated actively, causing BALP to be secreted into the blood. At the stage of calcification, osteocalcin (OC) appears. Bone formation is accelerated in OC-deficient

mice, suggesting that OC works as a suppressant of bone formation (Ducy, P. et al.: Increased Bone Formation in Osteocalcin-Deficient Mice; Nature, 382:448-452, 1996).

In the box of "Markers of bone formation" in Table 1, "(1) proliferation" corresponds to the phase of osteoblast proliferation and matrix formation, "(2) matrix formation" to the phase of matrix maturation, and "(3) mineralization" to the phase of calcification.

While there are various markers of bone formation, they frequently behave differently depending upon the state of the disease and it is important to realize specifically in which phase each marker appears.

There are also various markers of bone resorption and as with the markers of bone formation, metabolic products of type I collagen are currently drawing special attention. In type I collagen, collagen of a triple-stranded structure occurs crosslinked with pyridinoline and deoxypyridinoline, so when it is destroyed upon bone resorption, pyridinoline and deoxypyridinoline cross-links having various sizes of N- and C-terminal amino acids attached thereto are released into the blood.

The measurements of resorptive markers include that of cross-links alone (urinary pyridinoline and deoxypyridinoline that are measured as free entities), that of cross-links including C-terminal amino acids (CTx and ICTP), and that of cross-links including N-terminal amino acids (NTx). For generalized details about bone metabolic markers, see the review article by Calvo et al. (Calvo,

M.S. et al., Molecular Basis and Clinical Application of Biological Markers of Bone Turnover, Endocrine Rev., 17:333-368, 1996).

In bone metastasis, markers of bone metabolism behave somewhat differently than in metabolic bone diseases such as osteoporosis. Among formative markers, increased PICP and BALP are observed in bone metastasis of prostatic cancer which is a typical example of bone hardening metastases but there is no significant increase in the level of osteocalcin which rises in osteoporosis and other metabolic bone diseases. The mechanism behind these phenomena is not presently known. In breast cancer which involves bone metastasis of a mixed type, the levels of formative markers increase but not as much as in prostatic cancer. In lung cancer which involves many cases of bone metastasis of a dissolution type, there are no significant increases in the levels of formative markers.

Among resorptive markers, ICTP differs from the other bone metabolic markers in that it does not change greatly after menopause but it has been found to increase in bone metastasis of cancer. From the viewpoint of detecting bone metastasis, ICTP may be considered a good marker that is insensitive to enhanced bone resorption in the post-menopausal stage. The levels of resorptive markers increase not only in bone metastatis of lung cancer which is mostly of a dissolution type but also in bone metastasis of breast cancer which is mostly of a mixed type, as well as in bone metastasis of prostatic cancer which is of a

hardening type.

DISCLOSURE OF THE INVENTION

While the studies of bone metabolic markers have seen remarkable advances, comparisons of their advantages and limitations are presently far from being thorough and given many various markers of bone resorption and formation, one cannot tell for sure which markers are currently the best in diagnosis of bone metastasis.

In clinical diagnosis of bone metastasis, choice of a marker is entirely up to the discretion of each doctor and no technique has yet been established that allows for systematic monitoring of bone metastasis.

An object, therefore, of the present invention is to provide a tool capable of systematic monitoring of bone metastasis.

Under the circumstances, the present inventors conducted intensive studies with a view to developing a tool for systematic monitoring of bone metastasis and found that this object could be attained by combining a marker (formative marker) that reflects the activity of osteoblasts with a maker (resorptive marker) that reflects the action of osteoclasts. The present invention has been accomplished on the basis of this finding.

Thus, according to one aspect of the invention, there is provided a method of diagnosing bone metastasis of malignant tumors using a marker that reflects the activity of osteoblasts and a marker that reflects the action of osteoclasts.

According to another aspect of the invention, there is provided a method of evaluating the therapeutic effect of drugs using a marker that reflects the activity of osteoblasts and a marker that reflects the action of osteoclasts.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram showing how three different markers begin to appear as osteoblasts differentiate;

Fig. 2 is a graph showing the relationship between the efficacy of chemotherapy on patients with prostatic cancer involving bone metastasis and each of three markers, PICP, BALP and osteocalcin;

Fig. 3 is a graph showing the changes in a crossover index, osteocalcin/BALP, for patients with breast cancer; and

Fig. 4 is a graph showing the changes in the ICTP level during treatment of breast cancer.

BEST MODE FOR CARRYING OUT THE INVENTION

We now describe the findings which were the basis for accomplishing the present invention.

(1) Markers of bone formation as produced from osteoblasts:

As Fig. 1 shows, the differentiation of osteoblasts involves shifts in marker expression from PICP and PINP (type I procollagen peptides) through BALP (bone alkali phophatase) to osteocalcin (Stein, G.S. et al., FASEB J., 4:3111-3123, 1990).

(2) Reactions of BALP and osteocalcin to chemotherapy in patients with prostatic cancer and breast cancer who were

suffering from bone metastasis:

The levels of two formative markers, BALP and osteocalcin, differed with the state of bone metastasis, as demonstrated in the following two Examples.

Example 1

During the period from October 1994 to April 1996, the levels of formative markers were measured in 43 prostatic cancer patients with bone metastasis and 46 prostatic cancer patients without bone metastasis. Of the 46 prostatic cancer patients who apparently had no bone metastasis, 29 had received prostatectomy or radiation therapy and the remaining 17 were newly diagnosed patients who received prostatectomy or radiation therapy after bone scintigraphy and serum sampling. The patients without bone metastasis were aged 69 on average (ranging from 47 to 85 years old). The progress of prostatic cancer in these patients was as follows: four patients at stage A, 14 at stage B, 19 at stage C, and 9 at stage D1. Of the 43 patients with bone metastasis, 9 were newly diagnosed and received hormone therapy after bone scintigraphy and serum sampling. The remaining 34 patients had received positive treatments by hormone therapy and/or chemotherapy at various time intervals from the start of these treatments. The patients with bone metastasis were aged 69 on average (ranging from 53 to 83 years old).

After obtaining informed consent from all patients, blood samples were taken during bone scintigraphy and sera were separated and stored frozen at -40°C until analysis.

Serum BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was measured by immunoradiometric assay with a BGP-IRMA kit of Mitsubishi Chemical Corp.

The results are shown in Fig. 2, in which Z value is defined by (measured value - average for the patients with bone metastasis)/(standard deviation of a patient without bone metastasis). In Fig. 2, CR, flare, NC, IMP, new and PD have the following respective meanings.

CR : complete remission

flare : flare-up [the treatment was effective but the bone metastasis appeared to have progressed on a bone scan (scintigraphic) image].

NC : no change (no change was observed).

IMP : improvement (a sign of improvement was recognized).

new : diagnosed to have a new bone metastasis.

PD : progression of disease (the disease was found to have progressed).

For each of these patient groups, BALP and osteocalcin had the following Z values.

Z value of BALP

CR : 2.18

flare : 3.40

NC : 8.23

IMP : 2.39

new : 1.82

PD : 24.50

Z value of osteocalcin

CR : 1.30

flare : -0.18

NC : 0.04

IMP : 1.25

new : 0.08

PD : 0.05

Using these values, a crossover index (Z osteocalcin/Z BALP) was calculated for each patient group.

Crossover index

CR : 0.596

flare : -0.053

NC : 0.005

IMP : 0.523

new : 0.044

PD : 0.002

As is clear from the above data, BALP had a low Z value (2.18) in the CR group in which the treatments proved effective whereas it had a high Z value (24.50) in the PD group in which the disease worsened. On the other hand, osteocalcin had a high Z value (1.30) in the CR group but had a low Z value (0.05) in the PD group. The crossover index was 0.596 in the CR group but 0.002 in the PD group, with a marked difference being observed between the two groups. It can hence be concluded that the crossover index allows for both diagnosis of the progression of bone metastasis and evaluation of drug efficacy in the treatment of the disease.

Example 2

As in Example 1, the levels of formative markers (BALP and osteocalcin) were measured in a total of 850 patients with breast cancer, 644 of whom had bone metastasis and 206 having no bone metastasis. The patients with bone metastasis received chemotherapy or endocrine therapy targeted to the site of bone metastasis; they were classified into six groups, CR, NC, IMP, new and PD, according to the therapeutic efficacy achieved and the Z values of BALP and osteocalcin were determined in each group. On the basis of the measured Z values, a crossover index was calculated for each patient group.

After obtaining informed consent from all patients, blood samples were taken during bone scintigraphy and sera were separated and stored frozen at -40°C until measurement. Serum BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. Serum osteocalcin was measured by immunoradiometric assay with a BGP-IRMA kit of Mitsubishi Chemical Corp. The results are shown below.

<u>Z value of BALP</u>

CR : 0.741

NC : 1.514

IMP : 0.735

new : 2.021

PD : 5.041

<u>Z value of osteocalcin</u>

CR : 0.267

NC : 0.237

IMP : 0.039

new : -0.167

PD : 0.516

Crossover index

CR : 0.360

NC : 0.157

IMP : 0.053

new : -0.083

PD : 0.102

Fig. 3 is a graph showing the changes in crossover index as observed in the respective groups CR, NC, IMP, new and PD. Obviously, the crossover index for the group CR in which the treatments proved effective was 0.360 whereas the value for the group PD in which the disease worsened was 0.102, with a marked difference being observed between the two groups. It can hence be concluded that as in the case of prostatic cancer, the crossover index allows for both diagnosis of the progression of bone metastasis of breast cancer and evaluation of drug efficacy in the treatment of the disease.

The results of Examples 1 and 2 showed that the patients with bone metastasis in group CR who were effectively treated by drugs had high crossover index values whereas the patients with bone metastasis in group PD who changed for the worse without any therapeutic effect had low crossover index values. It was therefore supported that a crossover index between two osteoblast markers was extremely effective in evaluating the degree of amelioration

of bone metastasis (therapeutic efficacy of drugs).

In patients with prostatic cancer, the crossover index value of group CR was close to that of group IMP and so was the crossover index value of group NC to that of group PD; these data reflect the therapeutic efficacy for bone metastasis of prostatic cancer which was predominantly attributable to bone formation. The result from the patients with breast cancer was somewhat different in that the crossover index value of group NC was close to that of group PD whereas the crossover index value of group CR was not close to that of group IMP. The difference would have originated because breast cancer presents an image of bone metastasis to which bone dissolution is a more significant predisposing factor than bone formation. Therefore, in order to ensure that the progress of bone metastasis (the degree of aggravation) is diagnosed accurately, not only formative markers but also resorptive markers would have to be measured.

In Examples 1 and 2, the progress of bone metastasis of malignant tumors and the efficacy of their treatment by drugs were diagnosed by measuring the crossover index between osteocalcin which is a marker associated with the phase of calcification and BALP which is a marker associated with the phase of matrix maturation. The present inventors also verified that the progress of bone metastasis of malignant tumors and the efficacy of their treatment by drugs could be diagnosed by measuring the crossover index between osteocalcin which is a marker

associated with the phase of calcification and PICP and PINP which are a marker associated with the phase of osteoblast proliferation and matrix formation. Needless to say, osteocalcin can be replaced by any other markers that are associated with the phase of calcification, PICP or PINP can be replaced by any other markers that are associated with the phase of osteoblast proliferation and matrix formation, and BALP can be replaced by any other markers that are associated with the phase of matrix maturation.

In the past, several markers of bone formation have been identified and their levels have been individually measured to show that different markers were produced at different times depending on the stage in differentiation and maturation of osteoblasts. However, it has not been shown clearly which of the formative markers should be exclusively used as indices of bone metastasis to reflect the fact that "differentiation and maturation of osteoblasts are suppressed by bone metastasis of cancer". It was entirely unexpected from the prior art that the progress of bone metastasis and the efficacy of its treatment by drugs could be evaluated by the above-defined crossover index.

The bone to which cancer has metastasized is broken down by osteoclasts. While several markers are known to be capable of evaluating the bone resorption that accompanies bone destruction, the degree of bone metastasis (worsening of the disease) and the effect of therapy in suppressing

bone destruction can be evaluated definitely by identifying ICTP (type I collagen carboxy-terminal telopeptide) which is a collagen metabolite having a comparatively high molecular weight, validating ICTP as a reliable marker of bone resorption (see, for example, The Bone, Vol. 10, No. 3, pp. 111-118, 1996). Described below is an example showing the degree of bone metastasis and the effect of therapy in suppressing bone destruction.

Example 3: ICTP Level in Treatment of Breast Cancer

ICTP levels were measured in 23 patients with breast cancer who had received chemotherapy of bone metastasis by CAF regimen (C, cyclophosphamide; A, doxorubicin or Adriamycin; F, fluorouracil). The control group consisted of 9 patients with breast cancer who had no bone metastasis and received a CAF regimen as adjuvant therapy.

After obtaining informed consent from all patients, bone metabolic markers indicative of bone formation and resorption were measured. At the onset of CAF treatment and up to its end, blood samples were taken when bone scintigraphy was performed once a month and the sera were separated. The separated sera were stored frozen at -40°C until analysis. A formative marker BALP was measured by enzyme immunoassay with an ALKPHASE-B kit of Metra Biosystems. A resorptive marker ICTP was measured by radioimmunoassay with Orion Diagnostica. The serum CA 15-3 was measured by immunoradiometric assay with Centocor. The measured values were expressed in terms of the average and SE (standard error). A test of significance was carried

out by analysis of variance (ANOVA) according to the Bonferroni method.

The results are shown in Fig. 4, from which one can see that the ICTP values in the patients of group PD increased significantly over the ICTP values in the patients of group PR (partial response) and NC. The ICTP values of the patients of the "flare" group were significantly lower than those of the patients of group PD and substantially the same as those of the groups NC and PR without flare-up. The terms PD, flare and NC in Fig. 4 have the same meanings as in Example 1. PR means "partial therapeutic effect recognized".

No statistically significant difference was shown by the values of BALP and CA 15-3.

It is therefore concluded that by measuring the ICTP level, one can evaluate the degree of exacerbation of cancer metastasis to bone.

According to the findings in Examples 1 - 3, the amelioration of bone metastasis (therapeutic effect) and the degree of its exacerbation can be correctly diagnosed by monitoring two markers, one associated with osteoblasts and targeted to evaluation of therapeutic effect and the other associated with osteoclasts and targeted to evaluation of the worsening of the disease.

INDUSTRIAL APPLICABILITY

As described on the foregoing pages, the present invention provides a tool by which bone metastases caused by malignant tumors such as breast cancer, prostatic cancer

and lung cancer and the therapeutic efficacy of drugs for the cancers causative of such metastases can be diagnosed much more accurately than by the prior art methods.

CLAIMS

1. A method of diagnosing bone metastasis of malignant tumor using a marker that reflects the activity of osteoblasts and a marker that reflects the action of osteoclasts.

2. The method according to claim 1, wherein the marker that reflects the activity of osteoblasts is:

(1) a marker associated with the phase of osteoblast proliferation and matrix formation and a marker associated with the phase of calcification; or

(2) a marker associated with the phase of matrix maturation and a marker associated with the phase of calcification.

3. The method according to claim 1 or 2, wherein the marker that reflects the activity of osteoblasts is:

(1) PICP or PINP and osteocalcin; or

(2) BALP and osteocalcin.

4. The method according to any one of claims 1 - 3, wherein the marker that reflects the action of osteoclasts is a marker associated with bone type I collagen.

5. The method according to any one of claims 1 - 4, wherein the marker that reflects the action of osteoclasts is deoxypyridinoline and/or ICTP.

6. The method according to any one of claims 1 - 5, which is based on the value of a crossover index or the ratio between a marker associated with the phase of calcification and a marker associated with the phase of osteoblast proliferation and matrix formation and the measured value of the marker that reflects the action of

osteoclasts, or on the value of a crossover index or the ratio between a marker associated with the phase of calcification and a marker associated with the phase of matrix maturation and the measured value of a marker associated with bone type I collagen.

7. The method according to claim 6, which is based on the value of a crossover index between osteocalcin and PICP or PINP and the measured value of ICTP, or on the value of a crossover index between osteocalcin and BALP and the measured value of ICTP.

8. A method of evaluating the therapeutic efficacy of a drug using a marker that reflects the activity of osteoblasts and a marker that reflects the action of osteoclasts.

9. The method according to claim 8, wherein the drug is a cancer control therapeutic agent.

10. The method according to claim 8, wherein the drug is a bone resorption suppressant.

11. The method according to claim 8, wherein the drug is an endocrine therapeutic agent.

12. The method according to any one of claims 8 - 11, wherein the marker that reflects the activity of osteoblasts is:

(1) a marker associated with the phase of osteoblast proliferation and matrix formation and a marker associated with the phase of calcification; or

(2) a marker associated with the phase of matrix maturation and a marker associated with the phase of calcification.

13. The method according to any one of claims 8 or 12, wherein the marker that reflects the activity of osteoblasts is:

(1) PICP or PINP and osteocalcin; or

(2) BALP and osteocalcin.

14. The method according to any one of claims 8 - 13, wherein the marker that reflects the action of osteoclasts is a marker associated with bone type I collagen.

15. The method according to any one of claims 8 - 14, wherein the marker that reflects the action of osteoclasts is deoxypyridinoline and/or ICTP.

16. The method according to any one of claims 8 - 15, which is based on the value of a crossover index or the ratio between a marker associated with the phase of calcification and a marker associated with the phase of osteoblast proliferation and matrix formation and the measured value of the marker that reflects the action of osteoclasts, or on the value of a crossover index or the ratio between a marker associated with the phase of calcification and a marker associated with the phase of matrix maturation and the measured value of a marker associated with bone type I collagen.

17. The method according to claim 16, which is based on the value of a crossover index between osteocalcin and PICP or PINP and the measured value of ICTP, or on the value of a crossover index between osteocalcin and BALP and the measured value of ICTP.

ABSTRACT

Bone metastasis and the efficacy of drugs in the treatment of malignant tumors such as breast cancer, prostatic cancer and lung cancer that cause the bone metastasis are diagnosed using a marker that reflects the activity of osteoblasts and a marker that reflects the action of osteoclasts.

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添付公開書類
国際調査報告書
請求の範囲の補正の期限前の公開；補正書受領の際には再公開される。

(54)Title: METHOD FOR DIAGNOSING BONE METASTASIS OF MALIGNANT TUMOR

(54)発明の名称 悪性腫瘍の骨転移を診断する方法

(57) Abstract

Therapeutic effects of drugs on bone metastasis and cancer (mammary cancer, prostatic cancer, lung cancer, etc.) inducing bone metastasis are evaluated by using a marker reflecting the activity of osteoblasts and a marker reflecting the effect of osteoclasts.

Fig. 1

Osteoblast parameter
type I collagen
bone alkali phosphatase
osteocalcin
Differentiation and maturation of osteoblasts
mesenchymal stem cell
proosteoblast
osteoblast
mature osteoblast
bone cell

Translated

Fig. 2

Fig. 3

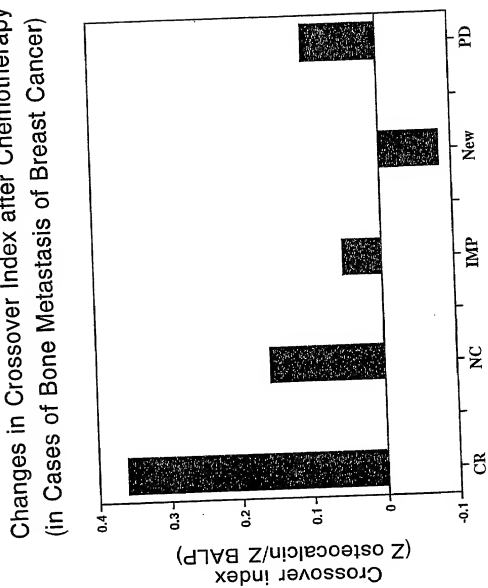
Changes in Crossover Index after Chemotherapy
(in Cases of Bone Metastasis of Breast Cancer)
Crossover index
(Z osteocalcin/Z BALP)
0.4
0.3
0.2
0.1
0
-0.1
CR
NC
IMP
New
PD

Fig. 4

Changes in ICTP Level during Chemotherapy by CAF Regimen
(in Patients with Breast Cancer)
ICTP ng/ml
20
15
10
5
0
PD
PR
PR accompanied by bone scan flare
NC
Start of treatment
1
2
3
4
5
6
Time (months)

PC/C-1-123

Page 1 of 2 Pages [x] Original [ ] Supplemental Atty. Docket:

Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD OF DIAGNOSING BONE METASTASIS OF MALIGNANT TUMORS

the specification of which (check one)

[ ] is attached hereto;

[ ] was filed in the United States under 35 U.S.C. §111 on _____, as U.S. Appln. No. _____*; or

[x] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/JP99/04480 filed August 20, 1999, entry requested on _____*; national stage application received U.S. Appln. No. _____*; §371/§102(e) date _____* (* if known)

and was amended on _____ (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 and 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day Month Year Filed)	YES	NO
236146/1998	Japan	21/8/1998	[x]	[]
			[]	[]

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or prior PCT application(s) designating the U.S. listed below, or under §119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from YUASA AND HARA as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

Title: METHOD OF DIAGNOSING BONE METASTASIS OF MALIGNANT TUMORS

U.S. Application filed _____, Serial No. _____

PCT Application filed August 20, 1999, Serial No. PCT/JP99/04480

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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